

Purification of antibodies using *Inceptum*[™] System.

From lab scale to production

Application note 032024-1.

Introduction

Purification of antibodies is often performed using affinity chromatography on protein G or protein A resin. Commonly used methods include spin-column purification and traditional liquid chromatography using low pressure system or an FPLC system configured for use of low pressure prepacked columns.

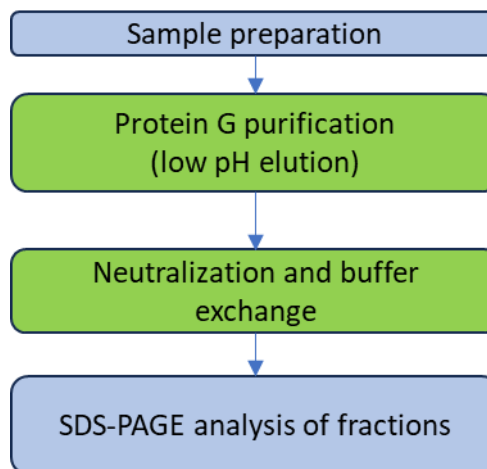
The spin column approach is relatively cheap and easy but requires significant hands-on time and prone to errors. While the method is easy to perform with common lab equipment, the purity of the resulting antibodies is not that great. Any scale up will require transition to a different system.

The use of a chromatography system typically provides higher purity but requires significant hands on time to setup the instrument and sufficiently trained personnel to be useful. Purification of multiple samples requires extensive cleaning of the system, especially if the requirements for potential cross-contamination are strict.

Inceptum[™] system utilizes a disposable purification cartridge. There is no need to do extensive cleaning of the system or setting up the fluidics. The system is ready for purification as soon as a new cartridge is plugged in. All reagents and purification columns are included in the cartridge.

Typical antibody purification protocol includes the steps outlines in Figure 1.

The complete protocol, including protein G affinity purification and buffer exchange in



PBS or customer-specified buffer is performed automatically in the IgG purification cartridge.

Figure 1. Workflow of IgG purification steps. Steps in green are performed in the *Inceptum*[™] IgG purification cartridge.

Methods

Small scale purification was performed using *Inceptum*[™] IgG purification cartridge and scale up utilized open cartridge. The fluidics diagram of both “IgG purification cartridge” and an “Open cartridge” are similar and shown in Figure 2. *Inceptum*[™] system

allows user to control each valve and pump manually or through a batch protocol (method) that is performed automatically. Valves V1 and V2 allow for sample injection and wash buffer through Pump B, valves V3 and V4 connect an elution buffer to Pump A. This configuration facilitates loading of practically unlimited sample volume through the main system pump. While such a use of the main system pump is a bad practice for most chromatography systems, *Inceptum™* utilizes disposable cartridges offering ease and convenience of such use without the

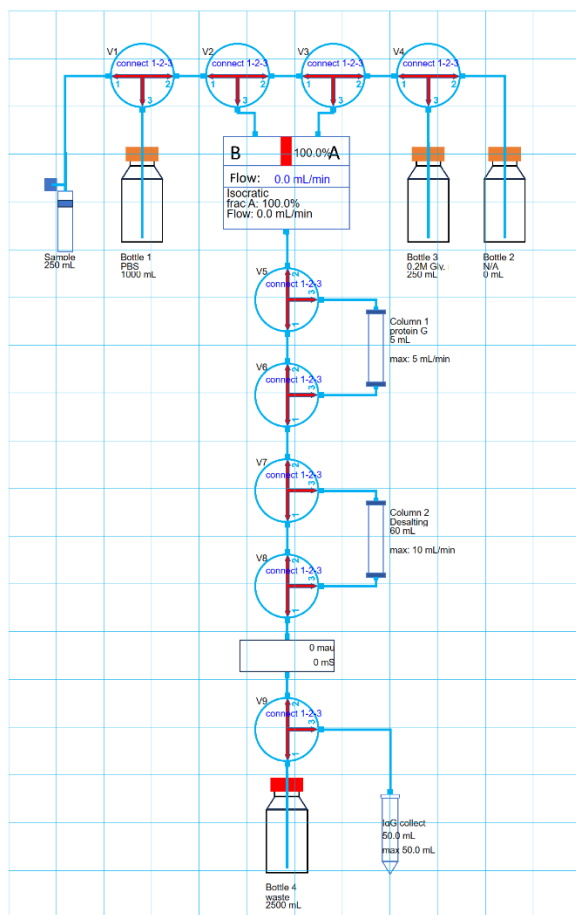


Figure 2. Fluidics diagram of the scale up purification.

detrimental effects on the main pump. Pairs of valves V5-V6, and V7-V8 are utilized for column switching. Each column can be independently switched in or out of the fluid flow, enabling 2D chromatography, or, easy buffer exchange in the case of IgG purification. Finally, valve V9 is a divert valve

that allow collection of the peak into sample collect vial or bottle and diverting the rest of the flow into waste.

Small scale purification.

The sample of normal goat serum was filtered through 0.2 um cellulose filter to remove particulates. One mL of the serum was injected into the *Inceptum™* IgG purification cartridge. The IgG purification cartridge contains a 5 mL injection loop, a larger volume of sample can be loaded by attaching sample reservoir to the injection port. Custom cartridges with a different injection loop volume are available.

Scale up purification.

For scale up, the sample was prepared similarly, and 5 mL was loaded through the sample input line of the open cartridge. A practically unlimited volume of the sample can be loaded in this configuration without any changes to fluidics. The fluidics connections of the open cartridge are shown in Figure 2. The purification method used for the scale-up purification is essentially the same as the one used for the 1 mL purification with adjustment for the sample volume.

Results

IgG purification using the *IgG purification cartridge* is shown in Figure 3. The automated process includes loading of the sample onto the 1 mL protein G column, washing to remove the unbound and non-specifically bound proteins, and elution using a low pH (0.1M Glycine, pH 2.5) buffer, typical of the protein G column purification. As the IgG peak is coming off the column, the flow is switched to the second, desalting, column to provide in-line buffer exchange. This way, the IgG are exposed to the low pH only a minimal time, reducing the possibility of the antibody degradation. The complete purification takes

less than 88 min and requires only about 5 min of hands-on time.

The scale up of the purification was performed using an open cartridge. Buffer solutions and columns were attached to the open cartridge externally. The use of the open cartridge allows significant scale up of the chromatography.

Cartridge: IgG purification cartridge
Sample: goat serum, filtered through 0.2 μm
Sample volume: 1 mL, undiluted.
Wash buffer: Phosphate Buffered Saline, pH7.4
Elution Buffer: 0.2M Glycine-HCl, pH2.5
Flow rate: variable, 0.5 – 5 mL/min depending on purification phase.
System: *Inceptum*TM Chromatography System
Detection: OD₂₇₅

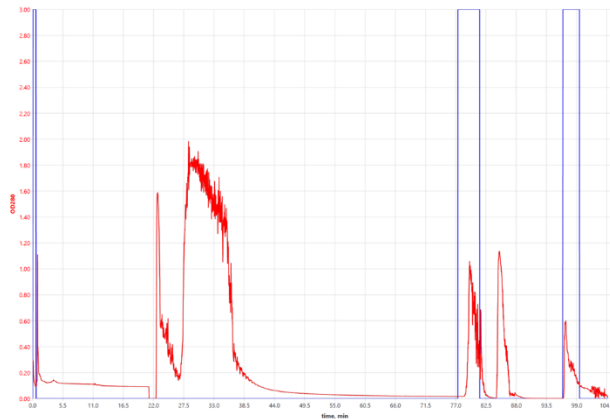


Figure 3. Purification of 1 mL (yield approx. 10 mg IgG) of goat serum using IgG purification cartridge. The chromatogram shows column preparation (0 – 22 min), Sample line priming (22-25 min), and sample load and column wash to remove unbound contaminants (27.5 – 77 min). Elution of the IgG from protein G column (77 – 82.5 min) was performed with low pH Glycine solution. As the IgG begins to elute, the flow is switched from protein G column to desalting column at approx.. 80 min. The purified IgG in phosphate buffered saline (PBS) are eluted between 85- 87 min. Column cleaning and system flush was performed after 88 min. The blue line represents concentration gradient, the red line represents OD₂₇₅.

Purified IgG were analyzed on SDS-PAGE to quantify purity. Gel images were captured using a 12 M pixel camera; quantitation was performed using GelAnalyzer software (rev 19.1 www.gelanalyzer.com). Purity of the IgG obtained in this purification was 94%.

Cartridge: Open cartridge
Sample: goat serum, filtered through 0.2 μm
Sample volume: 5 mL, undiluted.
Wash buffer: Phosphate Buffered Saline, pH7.4
Elution Buffer: 0.2M Glycine-HCl, pH2.5
Flow rate: variable, 1 – 10 mL/min depending on purification phase.
Column 1: PierceTM protein G cartridge, 5 mL
Column 2: Bio-ScaleTM Mini Bio-Gel® P-6 Desalting Cartridge, 50 mL
System: *Inceptum*TM Chromatography System
Detection: OD₂₇₅

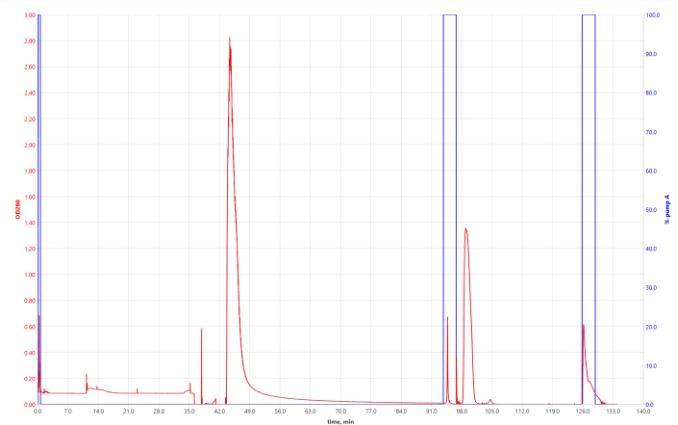


Figure 4. Scale-up purification of 5 mL of goat serum using open cartridge (yield approx. 50 mg IgG). The chromatogram shows column preparation (0 – 36.2 min), Sample line priming (36.2-38.2 min), and sample load and column wash to remove unbound contaminants (38.2 – 93.7 min). Elution of the IgG from protein G column (93.7 – 96.7 min) was performed with low pH Glycine solution. As the IgG begins to elute, the flow is switched from protein G column to desalting column at approx.. 94.6 min. The purified IgG in phosphate buffered saline (PBS) are eluted between 98.4- 100.5 min. Column cleaning and system flush was performed after 101 min. The blue line represents concentration gradient, the red line represents OD₂₇₅.

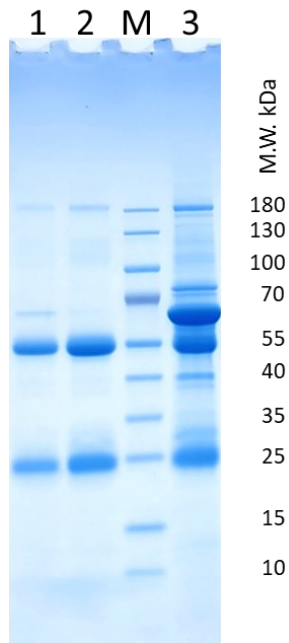


Figure 5. SDS-PAGE analysis of purified IgG. Samples were analyzed on Tris-Glycine 4-20% wedge gel (Invitrogen). Lane 1: small scale purification of IgG, Lane 2: scale-up purification of IgG, Lane M: molecular weight marker (PageRuler, ThermoFisher), Lane 3: starting material (goat serum). Densitometric analysis indicates purity of IgG is 94-95%.

Summary

Inceptum™ system utilizes disposable cartridges for chromatographic purification. An application-specific cartridges, such as *IgG purification cartridge*, and Open cartridges provide convenient routine headache-free purification of antibodies from variety of sources, and allow a clear scale-up path for manufacturing, including manufacturing in regulated environment.

Ordering information

Product	Catalog number
<i>Inceptum™</i> Chromatography system	I1001
<i>Inceptum™</i> , IgG purification cartridge	C1101
<i>Inceptum™</i> , open cartridge, standard	C1103

For ordering or additional information see: www.q-biotech.com